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REVIEW

IFN-α subtypes: distinct biological activities in anti-viral therapy

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During most viral infections, the immediate host response is characterized by an induction of type I IFN. These cytokines have various biological activities, including anti-viral, anti-proliferative and immunomodulatory effects. After induction, they bind to their IFN-α/β receptor, which leads to downstream signalling resulting in the expression of numerous different IFN-stimulated genes. These genes encode anti-viral proteins that directly inhibit viral replication as well as modulate immune function. Thus, the induction of type I IFN is a very powerful tool for the host to fight virus infections. Many viruses evade this response by various strategies like the direct suppression of IFN induction or inhibition of the IFN signalling pathway. Therefore, the therapeutic application of exogenous type I IFN or molecules that induce strong IFN responses should be of great potential for future immunotherapies against viral infections. Type I IFN is currently used as a treatment in chronic hepatitis B and C virus infection, but as yet is not widely utilized for other viral infections. One reason for this restricted clinical use is that type I IFN belongs to a multigene family that includes 13 different IFN-α subtypes and IFN-β, whose individual anti-viral and immunomodulatory properties have so far not been investigated in detail to improve IFN therapy against viral infections in humans. In this review, we summarize the recent achievements in defining the distinct biological functions of type I IFN subtypes in cell culture and in animal models of viral infection as well as their clinical usage in chronic hepatitis virus infections.

Abbreviations

alb-IFN, albuferon; APOBEC, apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like; CIFN, consensus IFN; DC, dendritic cells; FV, Friend retrovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; IFNAR, interferon-α/β-receptor; iNOS, inducible NOS; IP-10, IFN-γ-induced protein 10; IRF, IFN-regulatory factor; ISG, IFN-stimulated genes; MCMV, murine cytomegalovirus; MDA-5, melanoma differentiation-associated protein 5; Mx, myxovirus resistance protein; OAS, oligoadenylate synthetase; poly I : C, polyinosinic : polycytidylic acid; RIG-I, retinoic acid-inducible gene 1 protein; SVR, sustained virological response; TAK-1, TGF-activated kinase-1; TLR, toll-like receptors; TRIM, tripartite motifs; VSV, vesicular stomatitis virus; WHV, woodchuck hepatitis virus

The biology of type I IFNs

During a viral infection, the initial host response against invading and replicating viruses is the induction of type I IFNs. IFNs are a multigene family consisting of numerous IFN-α subtypes which all bind to their receptor (IFN-α/β-receptor, IFNAR) leading to the activation of the JAK and STAT signalling pathway resulting in the expression of several hundred genes, so called IFN-stimulated genes (ISG). These gene products have various functions such as anti-viral, anti-proliferative, anti-tumour or immunomodulatory activities. Some of these directly inhibit viral transcription and translation and thus immediately reduce viral loads (Clemens and Elia, 1997; Stark *et al.*, 1998). Others improve the host innate or adaptive immune response by activation of NK cells (Trinchieri *et al*., 1981; Salazar-Mather *et al*., 1996), up-regulation of proteins of the antigen presentation machinery (Epperson *et al*., 1992; Hermann *et al*., 1998), maturation of dendritic cells (DC) (Le Bon *et al*., 2003), and augmentation of CD8$^+$ T-cell (Honda *et al*., 2005; Le Bon *et al*., 2006a,b) and B-cell responses (Le Bon *et al*., 2001; Le Bon *et al*., 2006b).

The IFN response is initially induced by recognition of viral components such as RNA or DNA. RNA viruses are recognized by pattern recognition receptors, for example endosomal toll-like receptors (TLR3, 7/8) or cytosolic helicases [retinoic acid-inducible gene 1 protein (RIG-I) or melanoma differentiation-associated protein 5 (MDA-5)]. In contrast, DNA viruses can be recognized by endosomal TLR9, cytosolic DNA-dependent activator of IFN-regulatory factors, the DNA-binding protein IFI16 or other DNA sensors [reviewed in Keating *et al*. (2011)] In the case of TLR3, the sensing leads to downstream signalling via TLR adaptor molecule 1 and in the case of RIG-I or MDA-5 via IFN-β-promotor stimulator 1 resulting in the TAK-binding kinase 1-dependent activation of IRF3 (IFN-regulatory factor) or the TAK-1-dependent (TGF-activated kinase 1) activation of NFκB [reviewed in Akira and Takeda (2004)]. With the exception of TLR3, all TLRs recruit MyD88 upon activation, which results in TAK-1-dependent activation of NFκB or the phosphorylation of MAPK. Post activation, these factors translocate to the nucleus and bind to the IFN-β promotor leading to the expression of the 'primary' IFN genes IFN-β and IFN-α4 (Erlandsson *et al*., 1998; Marie *et al*., 1998). This expression is independent of IRF7, which is required for the subsequent induction of all other IFN-α subtypes. IFN-β and IFN-α4 bind to IFNAR in an autocrine loop and induce IRF7 expression, which in turn leads to the expression of other IFN-α genes (Sato *et al*., 1998) and the expression of various ISG. This results in an anti-viral state of the infected as well as neighbouring cells that is characterized by the expression of ISG encoding enzymes like PKR, oligoadenylate synthetase (OAS), myxovirus resistance protein (Mx), apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like (APOBEC) or tripartite motifs (TRIM), which directly inhibit viral replication. PKR recognizes double-stranded RNA, gets activated by dimerization and following auto-phosphorylation. It then phosphorylates the eukaryotic translational initiation factor 2 resulting in a translational stop (reviewed in Garcia *et al*., 2006). OAS also targets dsRNA as a cofactor, which results in the oligomerization of ATP through an unusual 2′,5′-phosphodiester linkage that is followed by activation of RNase L. This ribonuclease then degrades cellular and viral RNAs (Silverman, 2007). *Mx* genes encode for GTPases, which recognize viral nucleocapsids and restrict their localization and the subsequent viral replication within the cell (Haller *et al*., 2007). For retrovirus infections, it was shown that other restriction factors are important for the anti-viral activity induced by type I IFN. The IFN-induced genes – APOBEC3F/3G, TRIM5α and tetherin – show potent anti-retroviral activity by hypermutation of viral DNA, compromising the uncoating step of the virus or the release of viral particles, respectively (Sheehy *et al*., 2002; Stremlau *et al*., 2004; Neil *et al*., 2008).

Apart from its more direct anti-viral effects, IFN-α can strongly modulate innate and adaptive immune responses in the host. IFN-α enhances the proliferation, cytotoxicity and IFN-γ secretion of NK cells (Biron *et al*., 1984; Li *et al*., 1990; Hunter *et al*., 1997). It up-regulates the expression of major histocompatibility complexes class I and II on antigen presenting cells (Epperson *et al*., 1992; Hermann *et al*., 1998) and facilitates cross-presentation of viral antigens to CD8$^+$ T-cells by DCs (Le Bon *et al*., 2003), which enhances adaptive immune responses. IFN-α also augments the cytotoxicity of CD8$^+$ T cells and macrophages and enhances the antibody production by B cells (Le Bon *et al*., 2006b). Type I IFN plays a pivotal role in the activation of virus-specific T cells in lymphocytic choriomeningitis virus infection of mice because CD8$^+$ T cells require type I IFN for optimal clonal expansion (Kolumam *et al*., 2005).

Due to the various effects of type I IFN, viruses have evolved many strategies to efficiently overcome the host IFN-response (reviewed in Randall and Goodbourn 2008) . Thus, exogenous application of type I IFN or endogenous induction by IFN-inducing drugs could increase anti-viral activity and improve host immune responses and should therefore be useful for clinical treatment of several virus infections.

Type I IFN in the clinical treatment of viral diseases

Right after the discovery of type I IFN in 1957 (Isaacs and Lindenmann, 1957), researchers and physicians were interested in a possible clinical application of type I IFN. In 1980, IFN-α1, IFN-α2 and IFN-β were purified, cloned and sequenced, improving the clinical usage of type I IFN (Nagata *et al*., 1980).

Hepatitis C

To date, treatment with IFN-α2 is the backbone of therapy for acute and chronic hepatitis C (HCV). The rationale for the treatment of acute hepatitis C is to avoid the development of chronicity. Initially, it could be shown that a 24-week course of monotherapy with unpegylated IFN could cure more than 90% of the acutely infected patients (Jaeckel *et al*., 2001; Wiegand *et al*., 2004). More recent studies demonstrated that pegylated IFN (pegIFN) is equally effective (Santantonio *et al*., 2005; Wiegand *et al*., 2006). Currently, there are two pegIFN on the market: pegIFN-α2a and pegIFN-α2b.

The beneficial effects of IFN-α in chronic HCV were first reported in 1986 leading to its approval for clinical use in hepatitis C by the Food and Drug Administration in 1990. Initially, a course of IFN at a dose of 3 million units twice weekly for 48 weeks was recommended (NIH, 1997). However, it was associated with very limited rates of sustained virological response (SVR), in the range of 12–16%. The addition of ribavirin significantly improved response rates to the range of 35–45% (McHutchison and Poynard, 1999). From 2001 to 2011, the combination of pegIFN and ribavirin was the standard treatment of chronic hepatitis C. These treatment protocols resulted in SVR rates of approximately 70–90% in patients with genotypes 2 and 3, but only 40–50% in patients with other genotypes (Manns *et al*., 2001; Fried *et al*., 2002). Genotypes 2 and 3 patients received pegIFN and weight-adjusted ribavirin for 24 weeks. More recently, triple therapy including IFN, ribavirin and a protease inhibitor has been established leading to SVR rates of approximately 70% in naive genotype 1 patients (Poordad *et al*., 2011; Sherman *et al*., 2011).

Hepatitis B

In the 1970s, the first clinical trials with low-dose impure IFN-α against hepatitis B virus infection (HBV) showed promising results (Greenberg *et al*., 1976). In 1991, conventional IFN-α2b was the first drug approved for the treatment of HBV infection (Hoofnagle *et al*., 1988). Its major mechanism of action is the modulation of the immune system, although there is also a weak direct anti-viral effect. However, it was difficult to select patients and decide when to start treatment as well as when to stop it. Thus, the treatment was arbitrarily given for 16–24 weeks. PegIFN-α2a was approved in 2005. Since then, conventional IFN-α has been gradually replaced by pegIFN-α2a. The duration of pegIFN-α therapy was arbitrarily chosen using 48 weeks compared with 16–24 weeks for conventional IFN-α. Even with the extension of therapy duration to 48 weeks, it has been shown that the HBeAg seroconversion rate (33%), which correlates with SVR, is almost identical to that of conventional IFN-α as determined in a meta-analysis (32%). In addition, after 3 years of follow-up for HBeAg-negative patients with lower baseline HBV DNA levels, the rate of undetectable HBV DNA by PCR is only 18% (Marcellin *et al*., 2009).

New IFNs

Beside recombinant pegIFN-α, a variety of different forms of IFN-α are under development and clinical investigation. The recombinant fusion protein of IFN-α2b with human serum albumin, so called albuferon (alb-IFN), is under intense investigation. It was shown *in vitro*, that alb-IFN has the same anti-viral properties as IFN-α, and the induction of ISG is very similar to that of pegIFN-α2a and 2b (Liu *et al*., 2007). *In vivo* studies in cynomolgus monkeys revealed a prolonged half-life of alb-IFN in comparison with IFN-α (Osborn *et al*., 2002). The efficacy of alb-IFN was evaluated in clinical phase IIb studies with IFN-naive patients chronically infected with HCV, in which it was administered every 2 or 4 weeks (Zeuzem *et al*., 2008). The clinical studies showed that alb-IFN had similar anti-viral effects to pegIFN-α (Nelson *et al*., 2010; Zeuzem *et al*., 2010). However, in phase III studies, a higher incidence of serious pulmonary adverse events was observed in alb-IFN-treated patients, which resulted in the cessation of further development by Novartis and Human Genome Sciences in 2010.

Another recombinant IFN, which is under intense study, is the consensus IFN (CIFN). This IFN is an artificial cytokine that contains the most common amino acid at each position in the protein among all human IFN-α subtypes (Blatt *et al*., 1996). *In vitro* studies demonstrated a higher anti-viral activity against vesicular stomatitis virus (VSV) of CIFN compared with IFN-α2a or 2b (Ozes *et al*., 1992). Clinical studies with therapy-naive chronic HCV patients treated with CIFN plus ribavirin showed more SVR than with standard treatment (reviewed in Witthoft 2008) . However, due to its short half-life, CIFN has to be administered daily, which is an obvious disadvantage for clinical use.

IFN-α subtypes

Type I IFN belong to a multigene family consisting of multiple IFN-α subtypes but only one IFN-β, IFN-ε, IFN-κ, and IFN-ω (human) or limitin (mouse) (van Pesch *et al*., 2004). All 13 human IFN-α subtype genes are located on chromosome 9, whereas the murine genome encodes for 14 subtypes on chromosome 4. All type I IFN have similarities in structure, like the lack of introns or the length of the protein (161–167 amino acids), and their protein sequence is highly conserved (75–99% amino acid sequence identity) (Zwarthoff *et al*., 1985; Hardy *et al*., 2004). Interestingly, they all bind the same ubiquitously expressed receptor, called IFNAR, but they still differ in their biological activities. Explanations for their distinct functions come from studies reporting that the various human IFN-α subtypes all bind with different affinities to the IFNAR receptor subunits 1 and 2. One study compared the binding affinities of four different human IFN-α subtypes (IFN-α1, IFN-α2, IFN-α8 and IFN–α21) and IFN-β. The authors show that the K_D values of the IFN-α subtypes are all in the same range except IFN-α2 that had significantly lower affinities for the IFNAR1 ectodomain. (Jaks *et al*., 2007) The binding affinities to the IFNAR2 ectodomain were much higher than to IFNAR1. IFN-α1 showed the lowest affinity to IFNAR1 of all the subtypes tested. The authors also observed that IFN-β has the highest affinities for the ectodomains of both receptor subunits. Another study from 2011 basically confirmed these findings, but extended the previous work by analysing additional human IFN-α subtypes (Lavoie *et al*., 2011). They compared the binding affinities of all human IFN-α subtypes with respect to IFN-α2a. Only IFN-α10 and IFN–α17 had lower binding affinities than IFN-α2a, whereas all other human IFN-α subtypes showed a tighter binding to their receptor. The authors observed that the affinities were clearly associated with the anti-proliferative potency of the different IFN-α subtypes, but no correlation with the anti-viral activities of the subtypes was found, as determined in VSV and encephalomyocarditis virus neutralization assays. The differences in the receptor affinities can result in different downstream signalling cascades shown by phosphorylation of STAT molecules and MAPK (Cull *et al*., 2003). The authors show that murine IFN-α1, IFN-α2, IFN-α4 and IFN-α5 induced a tyrosine phosphorylation of STAT1, whereas tyrosine phosphorylation of STAT3 was only induced in response to IFN-α1. For IFN-α subtypes, it was also suggested that the quantity of the receptor on the surface of a specific target cell correlates with different biological activities of the subtypes indicating that abundant IFNAR expression might compensate for the weak binding affinity of certain IFN-α subtypes (Moraga *et al*., 2009). In addition to the varying binding affinities, differences in tissue-specific expression of the IFN-α subtypes or the receptor may influence biological activities. Moll and colleagues showed that the ISG mRNA induction upon stimulation with the different human IFN-α subtypes was different in fibroblasts than in endothelial cells and depended on the ISG analysed (Moll *et al*., 2011). The IFN-producing cell type and the type of infecting virus may also change the expression and action of different IFN-α subtypes (Baig and Fish, 2008; Easlick *et al*., 2010). For example, for SIV-infected rhesus macaques it was reported that different IFN-α subtypes (IFN-α1/13, IFN-α2, IFN-α4, IFN-α6 and IFN–α8) are rapidly expressed after an infection in lymphoid tissues such as tonsils, whereas in mucosal tissue, only a weak and delayed type I IFN response was observed (Easlick *et al*., 2010). Others have reported that individual IFN-α subtypes induce the expression of a specific pattern of ISG, which is consistent with the model of different receptor affinities as well as cell type-specific responses (Der *et al*., 1998; Grumbach *et al*., 1999; Cull *et al*., 2003; Leaman *et al*., 2003; Severa *et al*., 2006). A detailed list of the different biological activities, their induction of ISGs and their expression after stimulation with viral components is shown in Table 1 for the human IFN-α subtypes and in Table 2 for the murine subtypes. However, until now, no unique function has been attributed to any given subtype (Uze *et al*., 2007; Lavoie *et al*., 2011) and the redundancy of the IFN-α subtype system is also very poorly understood. This implies that more research on individual IFN-α subtypes has to be performed to define their specific anti-viral activities.

So far, an only very limited number of *in vitro* and *in vivo* studies using virus infection models have reported on the distinct anti-viral activities of specific IFN-α subtypes. *In vitro* studies demonstrated that IFN-α1, IFN-α4, IFN-α5, IFN-α6 and IFN-α9 mediate anti-viral activity against herpes simplex virus (Harle *et al*., 2002), whereas IFN-α11 and IFN-α4 were the best candidates in blocking the replication of mengovirus (van Pesch *et al*., 2004). *In vivo*, it was shown that during Friend retrovirus (FV) infection of mice, therapeutic treatment with IFN-α1, IFN-α4, IFN-α9 and IFN-α11 reduced the viral loads significantly, whereas IFN-α2, IFN-α5 and IFN-α6 could not inhibit viral replication (Gerlach *et al*., 2009; Gibbert *et al*., 2012). In DNA-vaccination studies with different IFN-α subtypes against murine cytomegalovirus (MCMV) infection, it was shown that vaccination with IFN-α1, IFN-α4 and IFN-α9 as adjuvants resulted in decreased viral loads after virus challenge in the muscle only, whereas vaccination with IFN-α6 inhibited MCMV replication in all the organs investigated. Two IFN-α subtypes (IFN-α2 and IFN-α5) were not able to reduce viral loads in these experiments (Cull *et al*., 2002). In contrast to MCMV infection, DNA-vaccination studies against influenza virus infection identified IFN-α5 and IFN-α6 to be the most efficient subtypes in reducing viral titres (James *et al*., 2007). Vaccination studies with adenoviral vectors encoding for the FV envelope or group-specific antigen (Gag) proteins in combination with different IFN-α subtypes as adjuvants resulted in strong immune protection of vaccinated mice against FV challenge after vaccination with IFN-α2, IFN-α4, IFN-α6 and IFN-α9, whereas the IFN-α subtypes IFN-α1, IFN-α5 or IFN-β did not improve protection against retroviral challenge (Bayer *et al*., 2011).

In most of these infection studies only the direct anti-viral effects of IFN-α subtypes were investigated, as indicated by a reduction of viral titres. This does not take into account any anti-viral effect of IFN-α induced by an immediate direct anti-viral response followed by a modulation of innate and adaptive immune responses against the virus. This dual role of type I IFNs has been shown for chronic HCV and HBV infection. During the first phase of IFN-α-treatment, a strong decline in viral loads was observed due to the direct anti-viral effects induced by IFN-α. However, the virus is usually not completely eliminated during this phase of treatment in infected patients (Neumann *et al*., 1998). During on-going treatment, viral loads further decrease slowly, which can finally result in a total clearance of HBV or HCV. These data suggest that in the first phase of IFN-α treatment, the rapid decline in viral loads is mediated by the induction of anti-viral enzymes. In the later phase of IFN-α treatment, modulation of innate and adaptive immune cells is required for viral clearance (Herrmann *et al*., 2003; Feld and Hoofnagle, 2005; Stegmann *et al*., 2010). We investigated the immunomodulatory effects of some IFN-α subtypes during Friend Retrovirus infection *in vivo*. IFN-α1 stimulated virus-specific cytotoxic T cells, and during treatment with IFN-α11 or IFN-α1, both are required for an optimal NK cell response (Gerlach *et al*., 2009; Gibbert *et al*., 2012). Others investigated the immunomodulatory effect of IFN-α2 on NK cells during treatment of HCV-infected patients (Ahlenstiel *et al*., 2011). This dual action, especially the unique immunomodulatory effects of IFN-α subtypes, should be investigated in detail for future treatments of viral infections.

Drugs that induce endogenous IFN-α production

The induction of type I IFN depends on the recognition of invading pathogens by TLRs and other sensing receptors. Artificial ligands for TLRs can be used to mimic infections and to induce IFN responses in the host. This can be beneficial for therapeutic treatment of infections to improve the host immune response and combat pathogen replication. Many studies with different TLR ligands were performed in cell culture or animal models for virus infections. Synthetic ligands for TLR3 and 9 (polyinosinic : polycytidylic acid (poly I : C) and CpG oligodeoxynucleotides respectively) were shown to be effective in treating viral infections like HIV, HBV, HCV, herpes virus or FV (McClary *et al*., 2000; Ashkar *et al*., 2004; Isogawa *et al*., 2005; Gill *et al*., 2006; Kraft *et al*., 2007; McHutchison *et al*., 2007; Trapp *et al*., 2009; Gibbert *et al*., 2010). We and others have shown in mice that the therapeutic effect of the TLR ligand used depends on the induction of type I IFN, as therapeutic treatment with the different TLR ligands is not effective in mice deficient in the type I IFN receptor ($IFNAR^{-/-}$) (McClary *et al*., 2000; Isogawa *et al*., 2005; Gill *et al*., 2006; Gibbert *et al*., 2010). For the hepadnavirus woodchuck hepatitis virus (WHV), it was shown that the stimulation of peripheral blood lymphocytes from acute and chronic WHV-infected woodchucks with the TLR3 ligand poly I : C reduced viral titres *in vitro* and *in vivo*, and this was due to the expression of specific woodchuck IFN-α subtypes (Lu *et al*., 2008). Ligands for TLR3 or TLR9 mainly induce type I IFN, which in turn induces the expression of various anti-viral genes and modulates the immune cell function. As most of the TLRs are expressed by DC, the therapeutic treatment with TLR ligands also results in the activation and improved antigen presentation of DC and thus further improves priming of T-cell responses (Lore *et al*., 2003).

Table 1

Biological activity of human IFN-α subtypes

IFN-α subtype	Induction	Differential biological effects (all *in vitro*)	Induced ISGs	References
α1/13			lower IP-10 induction compared with IFN-α2	Hilkens *et al.*, 2003
	In poly I : C or LPS-stimulated monocytes			Hillyer *et al.*, 2012
	By CpG and imiquimod in PBMC			Puig *et al.*, 2012
		Less potent against VSV compared with IFN-α8		Jaks *et al.*, 2007
		Lowest anti-viral activity against influenza A virus	Lowest activity to induce ISG mRNA compared with all other IFN-α subtypes	Moll *et al.*, 2011
	By HSV, NDV and RSV in PBMC			Loseke *et al.*, 2003
α2			High APOBEC3G, APOBEC3A, PKR and IDO induction	Vazquez *et al.*, 2011
			Strong induction of IP-10 and iNOS in DC but not in T cells; induction of IL-12Rβ2 in T cells but not DC	Hilkens *et al.*, 2003
	By CpG and imiquimod in PBMC			Puig *et al.*, 2012
	By HSV, NDV and RSV in PBMC			Loseke *et al.*, 2003
		High anti-viral activity against influenza A virus	Potent inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al.*, 2011
		Induces chemokinesis of T cells and T cell migration		Foster *et al.*, 2004
		low activity against human metapneumovirus		Scagnolari *et al.*, 2011
α4		Intermediate anti-viral activity against influenza A virus	Intermediate capacity to induce IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al.*, 2011
α5	Main subtype in the liver			Castelruiz *et al.*, 1999
		Stronger Stat1 and Tyk2 signaling than IFN-α2	High 2′-5′-OAS expression in hepatocytic cells	Larrea *et al.*, 2004
		highest anti-viral acitivity against VSV		Jaks *et al.*, 2007
			Intermediate capacity to induce IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al.*, 2011
		Strong anti-viral activity against human metapneumovirus		Scagnolari *et al.*, 2011
α6	Low in pDC			Szubin *et al.*, 2008; Hillyer *et al.*, 2012
	Low by HSV, NDV and RSV in PBMC			Loseke *et al.*, 2003
			Potent inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al.*, 2011
		Strong anti-viral activity against human metapneumovirus		Scagnolari *et al.*, 2011

Table 1
Continued

IFN-α subtype	Induction	Differential biological effects (all *in vitro*)	Induced ISGs	References
α7	Not induced by imiquimod-stimulation in pDC			Hillyer *et al*., 2012
	By CpG in PBMC			Puig *et al*., 2012
			Potent inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al*., 2011
α8	Weak induction in pDC			Hillyer *et al*., 2012
	By CpG in PBMC			Puig *et al*., 2012
	Intermediate induction by HSV, NDV and RSV in PBMC			Loseke *et al*., 2003
		Most effective in suppressing HCV replication		Koyama *et al*., 2006
			Potent inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al*., 2011
		Strong anti-viral activity against human metapneumovirus		Scagnolari *et al*., 2011
α10	In poly I : C-stimulated DC and CpG-stimulated macrophages			Hillyer *et al*., 2012
	By CpG in PBMC			Puig *et al*., 2012
		Most effective anti-viral activity against SFV and VSV		Yamaoka *et al*., 1999
			Potent inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al*., 2011
		Strong anti-viral activity against human metapneumovirus		Scagnolari *et al*., 2011
α14	In CpG-stimulated macrophages			Hillyer *et al*., 2012
	By CpG and imiquimod in PBMC			Puig *et al*., 2012
			Potent inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al*., 2011
α16	By CpG in PBMC			Puig *et al*., 2012
			Intermediate inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al*., 2011
α17		Less potent against human metapneumovirus		Scagnolari *et al*., 2011
α21	High in rubella virus-infected ECV304 cells			Mo *et al*., 2007
	high in poly I : C-stimulated DC			Hillyer *et al*., 2012
			High APOBEC3G induction	Vazquez *et al*., 2011
	By CpG in PBMC			Puig *et al*., 2012
			Intermediate inducer of IFIT1, CXCL10, CXCL11, ISG15 and CCL8	Moll *et al*., 2011
		Less potent against human metapneumovirus		Scagnolari *et al*., 2011

HSV, herpes simplex virus; IDO, indoleamine 2,3-dioxygenase; NDV, Newcastle disease virus; PBMC, peripheral blood mononuclear cells; pDC, plasmacytoid DC; RSV, respiratory syncytial virus.

Table 2

Biological activity of murine IFN-α subtypes

IFN-α subtype	Induction	Differential biological effects (*in vitro*[1]/*in vivo*[2])	Induced ISGs	References
α1		Anti-viral activity against FV; improves NK and $CD8^+$ T-cell response		Gerlach *et al.*, 2009[2]
	By influenza virus in L929 cells			Fung *et al.*, 2004[1]
	By coxsackie virus in heart tissue			Baig and Fish, 2008[2]
		Strong anti-viral activity against HSV-2; improves $CD8^+$ T-cell responses		Austin *et al.*, 2006[2]
		Anti-viral activity against HSV-1		Austin *et al.*, 2005[2]
		Strong anti-viral activity against MCMV		Yeow *et al.*, 1997[2]
	By reovirus in cardiac myocytes and fibroblasts	Anti-viral against reovirus	Induces ISG15 and IRF7 in cardiac myocytes and fibroblasts	Li and Sherry, 2010[1]
α2	By reovirus in cardiac myocytes and fibroblasts	Anti-viral against reovirus	Induces ISG15 and IRF7 in cardiac myocytes and fibroblasts	Li and Sherry, 2010[1]
α4		Strong anti-viral activity against mengovirus		van Pesch *et al.*, 2004[1]
		Anti-viral activity against FV		Gerlach *et al.*, 2009[2]
	By influenza A virus			Fung *et al.*, 2004[1]
	High by SeV and MHV-1 in L929 and L2 cells and high by MHV-1 in lymph nodes			Baig and Fish, 2008[1/2]
	By reovirus in cardiac myocytes and fibroblasts	Anti-viral against reovirus	Induces ISG15 and IRF7 in cardiac myocytes and fibroblasts	Li and Sherry, 2010[1]
α5		Strong anti-viral activity against influenza virus		James *et al.*, 2007[2]
		Strong anti-viral activity against HSV-2		Austin *et al.*, 2006[2]
	By reovirus in cardiac myocytes and fibroblasts	Anti-viral against reovirus	Induces ISG15 and IRF7 in cardiac myocytes and fibroblasts	Li and Sherry, 2010[1]
α6T		Strong anti-viral activity against influenza virus		James *et al.*, 2007[2]
α7/10				
α8/6	By influenza virus			Fung *et al.*, 2004[1]
	By reovirus in cardiac myocytes and fibroblasts			Li and Sherry, 2010[1]
α9		Anti-viral activity against FV		Gerlach *et al.*, 2009[2]
α11		Improves NK cell responses during FV infection; anti-viral activity against MCMV	Induces PKR and OAS-1a in splenocytes	Gibbert *et al.*, 2012[2]
	By influenza A virus			Fung *et al.*, 2004[1]
		Strong anti-viral activity against mengovirus; strong antiproliferative activity (B16 melanoma cells)		van Pesch *et al.*, 2004[1]
α12		Strong antiproliferative activity (B16 melanoma cells)		van Pesch *et al.*, 2004[1]
	Not induced by influenza virus or poly I : C stimulated L929 cells	Anti-viral activity against influenza A virus		Tsang *et al.*, 2007[1]
α13		Anti-viral activity against Theiler's virus, mengovirus, and VSV		van Pesch and Michiels, 2003
αA				
αB	By influenza A virus			Fung *et al.*, 2004

The nomenclature conforms to that used by van Pesch *et al.* (2004). MHV-1, mouse hepatitis virus-1; SeV, Sendai virus.

Also, ligands for TLR7 and 8 can be used therapeutically. They do induce type I IFN comparable with TLR3 or TLR9 ligands, show anti-viral potency and activate the host immune response (Horsmans *et al.*, 2005; Pockros *et al.*, 2007). Many clinical studies with agonists for TLR7 and TLR8 are under way, for example, against infections with HBV or HCV (refer to http://www.clinicaltrials.gov), which indicate their potential for future therapy of viral infections.

The application of some TLR ligands is already being used to treat cancer, allergies or viral infections. In addition, numerous studies have been performed to investigate the use of TLR ligands as adjuvants for vaccination. There are several vaccines available, which make use of monophosphoryl lipid A, a synthetic ligand for TLR4, to further improve the immune response against viruses (Fendrix® and Cervarix®, GlaxoSmithKline, Rixensart, Belgium; Supervax®, Dynavax Technologies, Berkley, CA, USA). Agonists for endosomal TLRs like TLR3, 7/8 and 9, which recognize viral nucleic acids, are promising candidates for the treatment of infectious diseases. Aldara® (3 M Pharma), which is already approved for the topical treatment of actinic keratosis, superficial basal cell carcinoma and external genital warts caused by human papilloma virus, modifies the anti-viral immune response. Aldara contains the TLR7 agonist imiquimod, which induces a local inflammatory response after binding to its receptor. The ligand recognition mediates the expression of pro-inflammatory cytokines and type I IFN in an IRF-7- and MyD88-dependent manner (Takaoka *et al.*, 2005; Honda *et al.*, 2006; Barchet *et al.*, 2008). This eventually results in the elimination of virus-infected as well as transformed cells.

A recent study by Hillyer and colleagues shows that *in vitro* treatment of human peripheral blood mononuclear cells, purified myeloid DCs, plasmacytoid DCs and monocytes with different TLR ligands resulted in the induction of specific IFN-α subtypes by the ligands tested (Hillyer *et al.*, 2012). This study reveals a coordinated ligand- and cell-specific expression of type I IFN subtypes, which might be useful for future treatment of viral infections with different IFN-α subtypes or their specific induction by exogenously applied TLR ligands.

Future perspectives

Therapeutic application of IFN-α or TLR agonists against various viral infections induces a direct anti-viral response in patients, which is followed by an enhanced immune response. One major problem of these therapies is the strong side effects of the agents. One idea might be to analyse the exact anti-viral and immunomodulatory activities of all the IFN-α subtypes present. The use of one specific IFN-α subtype might result in a more appropriate treatment, which would selectively combat the replicating virus and further improve the outcome of the therapy. In addition, a combination of the most potent anti-viral and immunomodulatory IFN-α subtypes might also be a new concept for effective treatment of viral infections.

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Conflict of interest

The authors state no conflict of interest.

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